revealed near-isometric contraction in this proximal zone, with dramatically elevated wall stress due to local thinning of the ventricular wall and elevated diastolic blood pressure. To directly investigate whether elevated wall stress triggers t-tubule disruption, we developed an *in vitro* model for culturing isolated rat left ventricle papillary muscles in a myobath system (0.5 Hz stimulation, 48 hours). Muscles were subjected to varying amounts of stretch to approximate wall stress values observed *in vivo*. Muscles exposed to low diastolic wall stress similar to that observed in sham-operated hearts (3.5-4.5 mN/mm^2), exhibited well-maintained t-tubule organization during culture (t-tubule fraction of cross-sectional area = 0.174 ± SE 0.007). Exposure to high wall stress (10-15 mN/mm^2) triggered marked t-tubule loss during culture (t-tubule fraction = 0.079 ± SE 0.012), and reduction in both peak and between-peak power in Fast-Fourier Transform analyses. In addition, cell size was observed to be markedly increased by elevated wall stress in comparison with muscles that were exposed to low wall stress (cross-sectional area = 1022.71 ± SE 48.00 μm^2 vs 564.41 ± SE 25.34 μm^2, $P < 0.05$). Thus, our data indicate that wall stress is an important regulator of both cellular geometry and t-tubular structure.

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Computational Model of Cross-Bridge Cycling and Force Generation to Explain the Effect of Metabolites on Cardiac Muscle Mechanics

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Despite extensive study over the past six decades the coupling of chemical reaction and mechanical processes in muscle dynamics is not well understood. We lack a theoretical description of how chemical processes (metabolite binding, ATP hydrolysis) influence and are influenced by mechanical processes (deformation and force generation). To address this need, a mathematical model of the muscle crossbridge (XB) cycle based on Huxley's sliding filament theory is developed that explicitly accounts for the chemical transformation events and the influence of strain on state transitions. The model is identified based on elastic and viscous moduli data from mouse and rat myocardial strips over a range of perturbation frequencies, and MgATP and Pi concentrations. Simulations of the identified model reproduce the observed effects of MgATP and MgADP on the rate of force development. Furthermore, simulations reveal that the rate of force re-development measured in slack-restretch experiments is not directly proportional to the rate of XB cycling. For these experiments, the model predicts that the observed increase in the rate of force generation with increased Pi concentration is due to inhibition of cycle turnover by Pi. Finally, the model captures the observed phenomena of force yielding suggesting that it is a result of rapid detachment of stretched attached myosin heads.

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From Contractile Non-Uniformities and Mechanical Instabilities to Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy (HCM), due to point mutations in genes for sarcomere proteins such as myosin, myosin-binding protein C and tropomyosin, occurs in 1/500 people and is the leading cause of sudden death in young individuals. The modified protein function leads, over years to decades, to secondary remodeling with hypertrophy, myofibrillar disarray and fibrosis associated with severe functional deterioration. Despite intense studies, it is poorly understood how rather moderate mutation-induced changes in protein function cause the long-term devastating effects. In ventricular wall hypertrophy due to pressure overload (e.g. hypertension), mechanical stress in the myocyte is believed to be major initiating stimulus for activation of relevant cell signaling cascades. It is here hypothesized that similar mechanisms activate hypertrofic cell-signaling in HCM but, in this case, non-uniformly over the left ventricle. Two possible underlying mechanisms are considered: i. contractile instabilities within each sarcomere (with more than one stable velocity for a given load) and ii. different tension generating capacities of cells in series. Statistical models for actomyosin cross-bridge function (1,2) are used to elucidate these mechanisms. Whereas non-uniformities between cells could have a range of different origins we show that contractile instabilities may result from mutations that produce increased cross-bridge stiffness. Further, the model simulations suggest that both mechanisms lead to enhanced local stretch of a fraction of the ventricular myocytes particularly during the overall isovolumetric phase of ventricular contraction. It is discussed how such local stretch may initiate cell signaling processes leading to the long-term severe pathology in HCM. Moreover, appropriate experimental tests of the proposed hypotheses are considered.

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Oxidative Stress Regulates Titin Elasticity by Affecting Ig-Domain Stability

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Background: The elasticity of titin is regulated through several mechanisms, including isoform switching and phosphorylation of unique spring elements (N2-Bus, PEVK). However, the titin springs consist mainly of immunoglobulin-like (Ig) domains, which are centrally involved in the molecular mechanism of titin elasticity. Passive force-regulating mechanisms targeting the Ig-domains of titin have not been described.
Methods and Results: We have elucidated a novel oxidative stress-related mechanism regulating muscle elasticity by altering the stability of titin-Ig domains (Alegre-Cebollada et al., Cell. 2014;156:1235-46). Using single-molecule AFM force spectroscopy, force measurements of isolated skinned human myocytes, and redox proteomics, we show that I-band Ig-domains of titin are weakened by oxidative modification of cryptic cysteines. We demonstrate that mechanical unfolding of these Ig domains exposes hidden cysteines, which now become accessible to disulfide bonding or S-glutathionylation in the presence of millimolar concentrations of oxidized glutathione (GSSG). In the AFM experiments, the cysteines of unfolded titin-Ig domains preferentially formed mixed disulfides with glutathione, which prevented the refolding of these domains. Oxidation by GSSG substantially reduced the passive tension of stretched human myocytes, and the effect was fully reversible with the incubation of reduced glutathione. Exposing perfused mouse hearts to oxidative stress (0.1 mM H2O2) revealed that Ig-domains from I-band titin are preferential targets of oxidation, as monitored using ICAT labeling/mass spectrometry.
Conclusions: Titin elasticity in striated muscle is modulated by oxidative stress through reversible weakening of Ig-domain stability via S-glutathionylation of buried cysteines. These titin Ig domains could also represent individual mechanosensors, whose mechanical properties determine mechano-chemical signaling processes in stressed myocytes.

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AB-Crystallin Binds to Titin Ig Domains and Increases Stiffness of Skinned Cardiac Trabeculae

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Abnormally stiff or compliant cardiac muscle is commonly observed following acute damage or disease. Cardiac stiffness is primarily modulated by the extracellular matrix protein collagen and intracellularly by the giant sarcomeric protein titin. However, it is not clear, particularly in the absence of fibrosis, how stiffness is altered in disease conditions. Recently, a mutation in the small heat shock protein αB-crystallin (R157H) was shown to cause inherited dilated cardiomyopathy. This abundant protein (3-5% of total soluble protein in the heart) is thought to bind titin and may regulate its stiffness. To test this, we measured the passive stiffness of skinned mouse trabeculae (with endogenous αB-crystallin extracted) by extending the sarcomere length from 2.0 to 2.6 μm in relaxing solution and measuring the resulting tension; addition of 1 mg/ml recombinant WT αB-crystallin significantly increased stiffness (linear Young's Modulus, extracted 31.2 ± 3.3; WT αB-crystallin 55.3 ± 8.7 mN/mm2). Passive stiffness at lower sarcomere lengths was increased more than at longer sarcomere lengths, indicating an increased contribution to force by titin relative to collagen. Interestingly, addition of the R157H mutant produced a significantly weaker effect. We show that αB-crystallin binds to fully folded titin Ig domains in vitro using native mass spectrometry and methyl trosy NMR. Our results indicate that αB-crystallin increases muscle stiffness not simply by stabilizing titin domains that have become unfolded during stretching, but by using a novel mechanism that is dissimilar to its regular function as a holdase chaperone. This work illustrates a further complexity in cardiac muscle regulation that may clarify disease pathogenesis and lead to additional therapeutic pathways.